Is a Diabetes Mellitus–Linked Amino Acid Signature Associated With β-Blocker–Induced Impaired Fasting Glucose?

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Background—The 5-amino acid (AA) signature, including isoleucine, leucine, valine, tyrosine, and phenylalanine, has been associated with incident diabetes mellitus and insulin resistance. We investigated whether this same AA signature, single-nucleotide polymorphisms in genes in their catabolic pathway, was associated with development of impaired fasting glucose (IFG) after atenolol treatment.

Methods and Results—Among 234 European American participants enrolled in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study and treated with atenolol for 9 weeks, we prospectively followed a nested cohort that had both metabolomics profiling and genotype data available for the development of IFG. We assessed the association between baseline circulating levels of isoleucine, leucine, valine, tyrosine, and phenylalanine, as well as single-nucleotide polymorphisms in branched-chain amino-acid transaminase 1 (BCAT1) and phenylalanine hydroxylase (PAH) with development of IFG. All baseline AA levels were strongly associated with IFG development. Each increment in standard deviation of the 5 AAs was associated with the following odds ratio and 95% confidence interval for IFG based on a fully adjusted model: isoleucine 2.29 (1.31–4.01), leucine 1.80 (1.10–2.96), valine 1.77 (1.07–2.92), tyrosine 2.13 (1.20–3.78), and phenylalanine 2.04 (1.16–3.59). The composite P value was 2×10⁻⁵. Those with PAH (rs2245360) AA genotype had the highest incidence of IFG (P for trend=0.0003).

Conclusions—Our data provide important insight into the metabolic and genetic mechanisms underlying atenolol-associated adverse metabolic effects.

Clinical Trial Registration—http://www.clinicaltrials.gov; Unique Identifier: NCT00246519

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Key Words: amino acids ▪ metabolomics ▪ pharmacogenetics

Hypertension and being overweight or obese have previously been identified as risk factors for diabetes mellitus and frequently coexist.1,2 Impaired fasting glucose (IFG), impaired glucose tolerance, and insulin resistance (IR), important early components of cardiometabolic dysfunction, are also prevalent in those with hypertension1 and significantly increase the risk for diabetes mellitus.3-5 β-blockers, although effective blood pressure (BP)-lowering agents, are associated with adverse metabolic effects, including hyperglycemia, IFG, and diabetes mellitus, all of which are associated with adverse cardiovascular consequences long term.6-8 Although the mechanistic underpinnings of β-blocker–associated adverse metabolic effects are incompletely understood, we have previously shown that risk for β-blocker–associated adverse metabolic effects, including hyperglycemia, IFG, and diabetes mellitus, all of which are associated with adverse cardiovascular consequences long term.
adverse metabolic effects is present in those with and without abdominal obesity.5

Identification of risk factors for diabetes mellitus has been a focus for decades, with the ultimate goal of developing strategies to delay or prevent onset of diabetes mellitus in those at highest risk. Based on data from observational and randomized clinical trials, clinical characteristics known to increase risk for drug-induced diabetes mellitus include age, ethnicity, race, body mass index, hypertension, and stroke, among many others.9

Metabolomics, a rapidly growing field that enables mapping of global biochemical changes associated with disease or treatment,10 has been used successfully to map pathways implicated in mechanisms of variation of response to drugs.11 Recently, metabolomics data from an observational study of the Framingham Offspring cohort identified a small cluster of essential amino acids (AAs), including baseline levels of 3 branched-chain amino acids (BCAA), isoleucine (Ile), leucine (Leu), and valine (Val), and 2 aromatic amino acids (AAA), phenylalanine (Phe) and tyrosine (Tyr), as metabolomic risk factors associated with a significant and independent increased risk for diabetes mellitus.12 This same 5 AA cluster has also been identified as a predictor of IR in young adults.13

Although the clinical characteristics that predict risk for drug-induced diabetes mellitus are similar to those that predict diabetes mellitus of other pathogeneses,14,15 currently no data are available assessing whether the same profile of BCAA and AAA that predicted diabetes mellitus and IR risk might also predict risk for drug-induced alterations in glucose status. Likewise, although BCAA transaminase 1, BCAT1, and phenylalanine hydroxylase, PAH, genes that catalyze BCAA and AA, respectively, have been previously associated with diabetes mellitus16 and IR,17 there are no data regarding the associations of single-nucleotide polymorphisms (SNPs) in these genes with drug-induced metabolic changes. Therefore, in a nested cohort from the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study, we used a targeted pharmacometabolomics-informed pharmacogenomics approach18 to investigate whether baseline levels of the previously identified BCAA and AAA and SNPs in genes in their catabolic pathways increase the odds of developing IFG, a phenotype associated with significantly increased risk of diabetes mellitus and cardiovascular disease after short-term exposure to atenolol.11

### Methods

PEAR is a prospective, randomized, parallel group, titration study undertaken to evaluate the pharmacogenomic determinants of the antihypertensive and adverse metabolic responses to atenolol and hydrochlorothiazide in participants with hypertension without a history of heart disease or diabetes mellitus. Details on study design and enrollment criteria have been previously published.20 The study is registered at clinicaltrials.gov, NCT00246519. The study site included participants from the Mayo Clinic, Rochester, MN, University of Florida, Gainesville, FL, Emory University, Atlanta, GA, and the Mayo Clinic, Rochester, MN. Because we are seeking to extend findings generated in a primarily white population, this analysis includes a subset of European American men and women with mild-to-moderate essential hypertension, between the ages of 17 and 65, selected for metabolomics profiling and with available genotype information within the atenolol arm of PEAR (Figure 1 in the Data Supplement). For metabolomics profiling, patients were divided into quartiles based on the diastolic BP response to atenolol monotherapy. Equal numbers of patients were selected randomly within each response quartile using the PROC SURVEYSELECT procedure (simple random sampling without replacement). Before baseline measurements, those receiving treatment for hypertension at enrollment had all antihypertensive drugs discontinued for a median of 27 (interquartile range [IQR] 19–34) days. Participants with IFG (glucose ≥ 100 mg/dL) at baseline were excluded from this analysis.

### PEAR Protocol

Written informed consent was obtained voluntarily from all participants, and institutional review board approval was obtained from all study sites, which included University of Florida, the Mayo Clinic, and Emory University. Atenolol was initiated at 50 mg and titrated to 100 mg based on BP≥120/70 mm Hg and tolerability. After 26 weeks on the final dose, response was determined, including BP measurements and laboratory-based assessments, and results described in this analysis come from this response assessment time point. PEAR is registered at ClinicalTrials.gov, #NCT00246519.

### Laboratory Measurements

At baseline and after completion of atenolol monotherapy, fasting blood samples were collected for glucose and insulin. Insulin sensitivity status was calculated using the homeostatic model assessment—IR (HOMA-IR).21

### Biochemical Assays

Glucose was measured in plasma on an Hitachi 911 Chemistry Analyzer (Roche Diagnostics) at the central laboratory at the Mayo Clinic, Rochester, MN, using spectrophotometry by an automated enzymatic assay. Plasma insulin was measured using the Access Ultrasensitive Insulin immunoassay system (Beckman Instruments). All samples were tested in duplicate, and data reported are means of the duplicate samples.

### AA Quantification

Plasma samples were transferred from the PEAR laboratory to the metabolomics core laboratory, University of California, Davis, where samples were extracted, derivatized, and analyzed as reported previously in great detail.22,23 Briefly, mass spectrometry used a Leco Pegasus IV time of flight mass spectrometer with 280°C transfer line temperature, electron ionization at –70 V, and an ion source temperature of 250°C. Mass spectra were acquired from m/z 85 to 500 at 20 spectra/sec and 1750 V detector voltage. Quantitative data were normalized to the sum intensities of all known metabolites and used for statistical investigation.

### Genotyping and Quality Control

To explore a potential functional mechanism underlying our observation that the 5 AA signature was associated with development of IFG after exposure to atenolol, we sought to investigate genes in common catabolic pathways. Because we had a limited population (n=184) with available genetic data and that did not have IFG at baseline, we used a candidate gene approach for this analysis. For the BCAAs, we chose to focus on BCAT1, which encodes the cytosolic form of the enzyme BCAA transaminase. This enzyme catalyzes the reversible transamination of branched-chain α-keto acids to branched-chain L-α amino acids for cell growth, and importantly, the aminotransferase step is the first step in the catabolic process for Ile, Leu, and Val. For the AAs, we chose to focus on PAH, which encodes the enzyme phenylalanine hydroxylase. Phenylalanine hydroxylase catalyzes the hydroxylation of the aromatic side-chain of phenylalanine to generate...
tyrosine and is the rate-limiting enzyme of the metabolic pathway that degrades excess phenylalanine.

Genotypes for SNPs in the BCA1 and PAH genes were obtained from the Omni1M quad GWAS Beadchip. The Omni1M quad is a GWAS chip that used the Infinium II assay, and genotypes were called using BeadStudio software and GenTrain2 calling algorithm. Procedures for quality Control and principal component (PC) analysis for determination of population substructure are described in detail in the Materials section in the Data Supplement. After QC procedures, minor allele frequency filter of >0.05, and linkage disequilibrium and monogenic SNP pruning, a total of 96 SNPs, 69 SNPs in BCA1, and 27 SNPs in PAH were analyzed in the genetic association study.

Primary Outcome

We examined the association between baseline plasma metabolite level of Ile, Leu, Val, Tyr, and Phe, as well as SNP genotypes and development of IFG, defined as a new occurrence of fasting glucose≥100 mg/dL, after treatment with atenolol monotherapy. After a 12-hour fast, glucose was measured in all participants at baseline, before initiation of atenolol, and again at the end of atenolol therapy. Mean duration of treatment with atenolol was 9 weeks. Participants with a fasting glucose ≥100 mg/dL at baseline were excluded from this study. Participants with a baseline fasting glucose <100 mg/dL and a fasting glucose ≥100 mg/dL at the end of atenolol treatment were considered to have developed IFG. Participants with a baseline fasting glucose <100 mg/dL and a fasting glucose that remained <100 mg/dL at the end of atenolol treatment were considered to have not developed IFG.

Statistical Analyses

Frequencies and percentages were calculated for categorical baseline characteristics and were compared by using χ² tests between those who did and did not develop IFG. Means and SDs or medians and interquartile ranges were calculated for continuous baseline characteristics and were compared using t tests or Wilcoxon rank-sum tests, respectively, between those who did and did not develop IFG. Change and percentage change in fasting glucose, baseline glucose, baseline insulin, and baseline AA levels were log-transformed because of non-normal distribution and compared across case/control groups. We used a regression model to assess the association between baseline AA level with the percentage change in glucose, adjusted for age, sex, body mass index, baseline glucose and insulin, and HOMA-IR in the entire cohort with metabolomics data available (n=150). Whether a participant developed IFG as a binary outcome variable was fitted by multiple logistic regression models. In separate analyses, each baseline plasma AA level was entered in the logistic model individually as a primary risk predictor. Because the distribution of those who did and did not develop IFG was significantly different by sex and previous research has shown that plasma AA levels differ significantly by sex,23 we performed a subgroup analysis where each baseline plasma AA level was performed separately by sex. The values of AA level were standardized (mean=0 and SD=1) so that the regression coefficients are interpreted as odds ratio (OR) per SD.23 ORs for AA level predicting IFG were estimated. For logistic regression analyses, we tested 4 separate models, (1) unadjusted; (2) adjusted for baseline age, sex, and body mass index; (3) model 2 plus fasting glucose and fasting insulin; and (4) model 3 plus HOMA-IR, to test the effect of IR. We evaluated the composite effect of the AAs by combining the individual P values from the model that included HOMA-IR. Because the BCAA and AAA were all strongly correlated (r=0.56–0.91, P<0.0001 for all comparisons), an extension to Fisher combination for correlated tests was performed for testing significance of the composite P value.24 For AA analyses, P values <0.05 were considered statistically significant.

For pharmacogenomics analyses, deviations from Hardy Weinberg Equilibrium (HWE) were assessed using Fisher Exact Test with α=0.05. The associations of the BCA1 and PAH SNPs and IFG were evaluated using logistic regression, adjusting for age, sex, baseline glucose, insulin, and HOMA (model 4 above), and the first 2 PCs for ancestry. To account for multiple comparisons, α levels for the SNP associations were set based on the total number of SNPs included in the analysis, which was 96: 0.05/96=0.0005. SNP genotype QC and genetic association analyses were performed in PLINK.27 All other analyses were performed by using SAS v9.3 (SAS Institute, Cary, NC).

Results

Pharmacometabolomic Analyses

Among the 234 participants randomized to the atenolol group in the PEAR study, pharmacogenetics profiling was performed on 150 (Figure I in the Data Supplement). Of those participants, 122 did not have IFG at baseline and are the focus of the pharmacogenetics analysis. A total of 24 participants developed IFG during an average 9 weeks of treatment with atenolol. Baseline characteristics of the participants included in the pharmacogenetics analysis are summarized in Table 1 and do not differ from the entire atenolol cohort (data not shown). Although those who developed IFG were more likely to be men, the cohort was similar in age, body mass index, and systolic and diastolic BP in those who did and did not develop IFG. Baseline fasting glucose was higher (Table 1), and change in glucose after treatment with atenolol was significantly greater among those who developed IFG than those who did not (mean 13.5 mg/dL versus 2.1 mg/dL, P<0.0001; Figure IIA in the Data Supplement). Median baseline plasma levels of Ile, Leu, Val, and Phe were significantly higher in those who developed IFG than those who did not, whereas there was no difference in baseline levels of Tyr (Figure IIB in the Data Supplement).

Baseline levels of all 5 AAs were significantly associated with percent change in glucose, even after adjustment for age, sex, body mass index, baseline glucose, and insulin and Table 1. Baseline Characteristics of the Cohort With Metabolomics Data, According to IFG Status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Developed IFG (n=24)</th>
<th>Did Not Develop IFG (n=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European American (n, %)</td>
<td>24, 100</td>
<td>98, 100</td>
</tr>
<tr>
<td>Women (n, %)</td>
<td>6, 25*</td>
<td>62, 63*</td>
</tr>
<tr>
<td>Current smoker (n, %)</td>
<td>3, 13</td>
<td>13, 13</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>52.5 (9)</td>
<td>49.6 (10)</td>
</tr>
<tr>
<td>Mean BMI, kg/m²</td>
<td>30.5 (4.5)</td>
<td>30.3 (6.2)</td>
</tr>
<tr>
<td>Mean waist circ, cm</td>
<td>98.5 (11)</td>
<td>96.6 (13)</td>
</tr>
<tr>
<td>Mean SBP, mm Hg</td>
<td>143 (9)</td>
<td>145 (10)</td>
</tr>
<tr>
<td>Mean DBP, mm Hg</td>
<td>92 (5)</td>
<td>92 (6)</td>
</tr>
<tr>
<td>Median fasting glucose, mg/dL</td>
<td>98 (86.0–98.0)</td>
<td>88.3 (83.5–91.5)*</td>
</tr>
<tr>
<td>Median fasting insulin, μU/mL</td>
<td>8.3 (4.8–11.2)</td>
<td>6.6 (4.9–9.6)</td>
</tr>
<tr>
<td>Median potassium, meq/L</td>
<td>4.5 (0.40)</td>
<td>4.3 (0.34)</td>
</tr>
<tr>
<td>Median HOMA-IR</td>
<td>1.86 (1.06–2.56)</td>
<td>1.42 (1.05–1.97)</td>
</tr>
</tbody>
</table>

Continuous variables that are normally distributed (age, body mass index [BMI], waist circumference [waist circ], systolic blood pressure [SBP], and diastolic blood pressure [DBP]) are presented as mean (SD). Continuous variables such as glucose, insulin, and homeostatic model assessment (HOMA) are not normally distributed and are presented as median (interquartile range). Categorical variables were presented as number and percentages. IFG indicates impaired fasting glucose; and IR, insulin resistance.

*P value<0.05 by using χ² or Wilcoxon rank-sum test to compare frequency between those who did and did not develop IFG.
HOMA-IR. The beta coefficients of the baseline 5 AAs are shown in Table 2.

**Association of Amino Acids With Impaired Fasting Glucose**

Association between baseline AA level (per SD increment) and odds for development of IFG for models 1 to 4 in the overall cohort is summarized in Table 1 in the Data Supplement. Results from Model 4 showed the following ORs and 95% confidence interval for IFG: Ile 2.29 (1.31–4.01), Leu 1.80 (1.10–2.96), Val 1.77 (1.07–2.92), Tyr 2.13 (1.20–3.78), and Phe 2.04 (1.16–3.59; Figure 1). When the $P$ values from the individual AAs were combined, the composite $P$ value was $2 \times 10^{-5}$ for Model 4, indicating a strong association between this 5 AA metabolite profile and odds for IFG after atenolol treatment. In subgroup analysis of odds for IFG according to sex, with the covariates from Model 4, odds for IFG after atenolol treatment in women (n=18 developed IFG and n=62 did not develop IFG), odds for IFG in men (n=18 developed IFG and n=62 did not develop IFG), the ORs ranged from 1.18 to 3.67; however, none of the associations reached statistical significance likely because of the small number of women who developed IFG. In men (n=18 developed IFG and n=36 did not develop IFG), the ORs ranged from 1.75 to 2.47, and baseline levels of Ile, Tyr, and Phe were associated with significantly increased adjusted odds for developing IFG. Data summarizing adjusted odds for IFG according to sex are presented in Table II in the Data Supplement.

**Pharmacogenetic Analysis**

Patients with the PAH rs2245360 AA genotype had the highest incidence of IFG (41.7%) compared with patients with AG genotype (15.2%) and GG genotype (7.4%), $P$ for trend=0.0003, which achieved statistical significance (Figure 2). In analyses of odds per allele for developing IFG after atenolol monotherapy, PAH rs2245360 had adjusted ORs of 3.51, 95% confidence interval 1.62 to 7.63, $P=0.0015$. None of the BCAT1 SNPs tested achieved Bonferroni-corrected statistical significance for development of IFG.

**Discussion**

We have shown, for the first time, that baseline plasma levels of Ile, Leu, Val, Tyr, and Phe, as well as a gene in a catabolic pathway, are strongly associated with increased odds of developing IFG in participants with hypertension, treated relatively short term (9 weeks) with atenolol, a commonly prescribed β-blocker. Importantly, IFG is an independent predictor of diabetes mellitus, and adding measures of anthropometrics and IR status into prediction models are often more informative for assessing diabetes mellitus risk. Here, we show that baseline levels of 4 of the 5 AAs investigated are significantly different in those who do and do not develop IFG, and that the AAs can further inform a prediction model for drug-induced IFG, an early metabolic risk phenotype in the diabetes mellitus continuum. Our data extend recent findings from the Framingham Offspring study, an observational study primarily in whites, which showed a strong association between these AAs and incident diabetes mellitus.

Overweight, obesity, glucose intolerance, and IR have been closely linked through many important biochemical and regulatory pathways. Knowledge that higher blood concentrations of Ile, Leu, Val, Tyr, and Phe are elevated in people with obesity, IR, or diabetes mellitus is not new. The continued rise in prevalence of risk conditions for diabetes mellitus in the last 2 decades has increased the need to better understand all relevant underlying pathways. Recent developments in metabolite profiling or metabolomics have provided insight into additional biochemical pathways that play an important role in glucose and insulin regulation. In a study comparing lean and obese individuals, BCAA and AAA were recently recognized as a metabolite cluster strongly associated with insulin sensitivity. Additionally, BCAA have been associated with coronary artery disease and diabetes mellitus. We have further confirmed BCAA as a significant predictor for atenolol-induced IFG in a population of middle-aged, otherwise healthy individuals with hypertension, providing additional evidence for its use as an important risk biomarker. In an analysis stratified by sex, Huffman et al showed that a cluster of large neutral AAs (Leu/Ile ratio, Val, Phe, Try, proline, and histidine) along with uric acid was a significant independent predictor of insulin sensitivity in both men and women. In our subgroup analysis evaluating the 5 AA signature stratified by sex, we confirmed an association with increased odds for IFG in men and saw similar, although nonsignificant, trends in women. Our inability to confirm an association in women is likely because of the small number of women who developed IFG in our study, this warrants further investigation in a larger cohort of women treated with atenolol.

Importantly, evidence also suggests that these AAs may play a causal role in metabolic dysregulation. A study evaluating the effect of a high-fat diet with or without BCAA supplementation in rats demonstrated that the high-fat plus BCAA diet resulted in a higher rate of IR than the high-fat diet alone. Similarly, a small study of healthy men who were infused a solution containing 18 AAs showed that AAs impair both insulin-mediated suppression of glucose production and insulin-stimulated glucose disposal in skeletal muscle. Together, these data suggest that these metabolites contribute to the development of metabolic dysregulation and are not just innocent bystanders in the metabolic disease continuum. Our data from a relatively overweight/obese hypertensive population suggest that treatment with atenolol may be another environmental exposure that has interactions among BCAA, AAA, and glucose regulation, as we observed significantly increased odds for IFG, an important early predictor for diabetes mellitus, associated with all AA tested.

**Table 2. Association Between Baseline and Percentage Change in Glucose After Treatment With Atenolol**

<table>
<thead>
<tr>
<th>Baseline AA</th>
<th>Ile</th>
<th>Leu</th>
<th>Val</th>
<th>Tyr</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>β Coefficient (SE)</td>
<td>0.31 (0.08)</td>
<td>0.28 (0.08)</td>
<td>0.24 (0.08)</td>
<td>0.21 (0.068)</td>
<td>0.18 (0.08)</td>
</tr>
<tr>
<td>$P$ Value</td>
<td>0.0003</td>
<td>0.001</td>
<td>0.004</td>
<td>0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

β coefficients were from multiple regressions adjusted for baseline glucose, baseline insulin, homeostatic model assessment–insulin resistance, sex, age, and body mass index. Ile indicates isoleucine; Leu, leucine; Phe, phenylalanine; SE, standard error; Tyr, tyrosine; and Val, valine.
linkage between the 12q22-24.2 region (PAH) and acute insulin response to glucose was observed. Linkage to fasting insulin has previously been reported near 12q23 in the Amish as well. Our observation of a significant difference in the development of IFG according to PAH rs2245360 genotype suggests this gene may play an important functional role in metabolic risk.

β-adrenergic blockers have long been recommended as first-line therapy for the treatment of hypertension, especially in patients with coronary artery disease. However, β-blockers have been implicated in altering glucose homeostasis, primarily through the inhibition of pancreatic insulin secretion and promoting IR. β-receptor selectivity seems to play a role in the degree of downstream metabolic effects, which include not only glucose increases but also weight gain and dyslipidemia. Nonselective and higher dose selective agents result in the largest adverse metabolic changes. Newer β-blockers including nebivolol and carvedilol seem to minimally affect glucose homeostasis and improve insulin sensitivity. Although the mechanisms underlying this differential effect on glucose remain unclear, they likely extend beyond β-blockade. Atenolol, the β-blocker used to treat participants in PEAR, is cardioselective, and the 100 mg dose used is the dose typically used to treat hypertension. We observe that AAs and PAH, a gene previously associated with insulin sensitivity, are also associated with atenolol-associated IFG. These data suggest the underlying mechanisms of drug-induced and primary dysmetabolism may be the same. Drugs may be an environmental trigger in patients with metabolic risk factors.

There are a few limitations of our study that are worthy of mention. First, although we targeted our analysis on a metabolite cluster previously identified for risk of diabetes mellitus in a population of whites, we performed our analyses only in a cohort of European Americans. Because our cohort is relatively small, particularly for analyses by sex, these results should be replicated in other populations with hypertension, untreated and treated with β-blockers and other drugs that possess adverse metabolic risks, for confirmation. Second, although we did observe a significant association with IFG in our cohort treated with atenolol, we cannot exclude that other metabolites are also playing a role. Third, our findings were identified among European American individuals with hypertension treated with atenolol and as such are only generalizable among similar patient populations. However, use of β-blockers in general, and atenolol specifically, is highly prevalent. In 2010, >36 million prescriptions were filled for atenolol or atenolol combinations in the United States. Finally, further investigation is warranted to (1) determine whether this 5-AA signature is causal in the development of IFG or is simply a marker of IR and impaired beta cell function, and (2) confirm our pharmacogenetic association and extend this finding to other antihypertensive agents associated with hyperglycemia, including thiazide diuretics and other race and ethnic groups at high risk for metabolic dysfunction.

In conclusion, we have extended the previous findings associating BCAA and AAs with incident diabetes mellitus to atenolol-induced IFG development. Our findings are important as they suggest novel biomarkers for the identification of those individuals at risk of developing antihypertensive treatment-induced diabetes mellitus. They may also provide insights to help better understand the mechanisms of β-blocker–induced dysglycemia. Although pharmacogenomics has shown to be informative with regard to drug–gene
interactions for β-blocker BP response, this study rep-resents one of the first to use a targeted pharmacometabolomics investigation combined with a pharmacogenomics investigation informed by the pharmacometabolomic findings for the antihypertensive drug–induced dysglycemia phenotype. With the prevalence of overweight, obesity, IR, hypertension, and diabetes mellitus on the rise not only in the United States but worldwide, understanding as many aspects of the mechanistic underpinnings of drug-induced adverse metabolic effects as possible is important. Pharmacometabolomics is a new but rapidly growing field that has promise in defining pathways implicated in mechanisms of variation of response to therapies and complements information gained from a pharmacogenomics approach. Together, they may be of immense value as the focus for drug therapy moves toward a personalized approach.

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This study was presented at the American Heart Association 2012 Scientific Sessions meeting in Los Angeles, California, in abstract form.

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Disclosures
R. Kaddurah-Daour holds patents in the metabolomics field. The other authors report no conflicts.

References

CLINICAL PERSPECTIVE

Although it has long been known that >100 commonly prescribed medications can adversely affect glucose levels, the underlying mechanisms associated with this dysglycemia are not well understood. Our observation that a 5-amino acid signature previously associated with diabetes mellitus is also strongly associated with atenolol-induced impaired fasting glucose, even after adjustment for standard biochemical measures of insulin resistance, suggests this to be an important diabetes mellitus risk factor. With diabetes mellitus prevalence continuing to increase, recent emphasis on early identification of those at greatest risk has been an important prevention strategy. Metabolomic signatures such as the 5-amino acids we describe may become a biomarker, which could be used in the clinical setting to identify individuals at increased risk for diabetes mellitus and to recognize those who would benefit from treatment with medications not associated with dysglycemia, where possible. Results from this study provide incentive to test the clinical use of this strategy.
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In the article by Cooper-DeHoff et al, “Is Diabetes Mellitus–Linked Amino Acid Signature Associated With β-Blocker–Induced Impaired Fasting Glucose?”, which appeared in the April 2014 issue of the journal (*Circ Cardiovasc Genet*. 2014;7:199–205), there was an error in the title.

It should read: “Is a Diabetes Mellitus–Linked Amino Acid Signature Associated With β-Blocker–Induced Impaired Fasting Glucose?”, with an “a” before “Diabetes”.

This has been corrected online. The author regrets the error.
Supplement Methods

Genotyping and Quality Control

Participants were excluded if sample genotype call rates were below 95% and SNPs were excluded if genotype call rates were below 90%. The genotype data were not reclustered after QC filters, but the genotype and sample call rate was recalculated after QC. Sample contamination was detected by checking gender mismatches using X chromosome genotype data and cryptic relatedness was estimated by pairwise identity-by-descent (IBD) analysis implemented using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/).\(^1\) Heterozygosity was also assessed using PLINK, by estimating the inbreeding coefficient, F. After the QC procedures, the total SNP call rate in the remaining individuals was 99.519%. Hardy-Weinberg equilibrium was assessed using chi-square tests with one degree of freedom.

To address the issue of population substructure and admixture in our racially and ethnically diverse population, a Principal Component Analysis (PCA) was performed in all subjects on a linkage disequilibrium (LD) pruned dataset using the EIGENSTRAT method.\(^2\) Race/ethnic groups were confirmed with PCA clustering results. If race/ethnic category disagreed strongly, patients were re-categorized to reflect the PCA result, considered to better reflect genetic ancestry. The top principal components (PCs 1-2) that provided the best separation of ancestry clusters were selected to be included as covariates for analysis.
Supplement References


## Supplement Table 1

Association between baseline amino acid level (per standard deviation) and odds for developing impaired fasting glucose following treatment with atenolol

<table>
<thead>
<tr>
<th></th>
<th>Model 1 OR (95% CI), p value</th>
<th>Model 2 OR (95% CI), p value</th>
<th>Model 3 OR (95% CI), p value</th>
<th>Model 4 OR (95% CI), p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ile</td>
<td>1.91 (1.23-2.96), 0.004</td>
<td>1.64 (1.03-2.62), 0.037</td>
<td>2.28 (1.29-4.01), 0.004</td>
<td>2.29 (1.31-4.01), 0.0034</td>
</tr>
<tr>
<td>Leu</td>
<td>1.69 (1.10-2.58), 0.017</td>
<td>1.46 (0.93-2.28), 0.101</td>
<td>1.76 (1.07-2.88), 0.025</td>
<td>1.80 (1.10-2.96), 0.019</td>
</tr>
<tr>
<td>Val</td>
<td>1.68 (1.09-2.59), 0.020</td>
<td>1.54 (0.97-2.45), 0.068</td>
<td>1.76 (1.06-2.91), 0.027</td>
<td>1.77 (1.07-2.92), 0.025</td>
</tr>
<tr>
<td>Tyr</td>
<td>1.64 (1.06-2.54), 0.028</td>
<td>1.68 (1.05-2.71), 0.032</td>
<td>2.03 (1.16-3.56), 0.014</td>
<td>2.13 (1.20-3.78), 0.010</td>
</tr>
<tr>
<td>Phe</td>
<td>1.62 (1.03-2.54), 0.037</td>
<td>1.67 (1.03-2.71), 0.039</td>
<td>2.01 (1.14-3.54), 0.016</td>
<td>2.04 (1.16-3.59), 0.014</td>
</tr>
</tbody>
</table>

Model 1 = unadjusted, model 2 = model 1+ age, sex, BMI, model 3 = model 2 + baseline glucose and baseline insulin, model 4 = model 3 + HOMA IR. Each amino acid level is included in the models as a continuous variable. Values are odds ratios per standard deviation (95% confidence intervals) and p values for impaired fasting glucose from an unadjusted logistic regression model and conditional logistic regression models adjusted for baseline fasting glucose and baseline fasting insulin or baseline fasting glucose and baseline fasting insulin. Ile=isoleucine, Leu=leucine, Val=valine, Tyr=tyrosine, Phe=phenylalanine
Supplement Table 2

Association between baseline amino acid level (per standard deviation) and odds for developing impaired fasting glucose following treatment with atenolol according to gender

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=68 (6 developed IFG and 62 did not develop IFG)</td>
<td>n=54 (18 developed IFG and 36 did not develop IFG)</td>
</tr>
<tr>
<td></td>
<td>OR (95% CI), p value</td>
<td>OR (95% CI), p value</td>
</tr>
<tr>
<td>Ile</td>
<td>3.67 (0.84-16.35), 0.084</td>
<td>2.06 (1.09-3.89), 0.025</td>
</tr>
<tr>
<td>Leu</td>
<td>1.90 (0.51-7.05), 0.339</td>
<td>1.75 (0.97-3.16), 0.063</td>
</tr>
<tr>
<td>Val</td>
<td>1.36 (0.52-3.58), 0.531</td>
<td>1.85 (0.97-3.54), 0.062</td>
</tr>
<tr>
<td>Tyr</td>
<td>1.18 (0.38-3.66), 0.780</td>
<td>2.47 (1.15-5.32), 0.020</td>
</tr>
<tr>
<td>Phe</td>
<td>1.36 (0.46-4.05), 0.576</td>
<td>2.25 (1.07-4.72), 0.032</td>
</tr>
</tbody>
</table>

Values are odds ratios (OR) per standard deviation (95% confidence intervals [CI]) and p values for impaired fasting glucose from logistic regression models adjusted for age, body mass index, baseline fasting glucose, baseline fasting insulin and HOMA IR. Each amino acid level is included in the models as a continuous variable. Ile=isoleucine, Leu=leucine, Val=valine, Tyr=tyrosine, Phe=phenylalanine
SUPPLEMENT FIGURE LEGENDS

**Figure 1.** Flow diagram of participants included in the analyses.

**Figure 2.** In the 122 participants with metabolomics data and without impaired fasting glucose at baseline, **A.** Change in glucose following treatment with atenolol according to those who did and did not develop IFG, **B.** Baseline amino acid levels according to those who did and did not develop IFG for Isoleucine, Leucine, Valine, Tyrosine and Phenylalanine. The data in B are presented as ion counts (measurement unit). Horizontal bars are median and 25\textsuperscript{th} and 75\textsuperscript{th} percentile. Whiskers are 5\textsuperscript{th} and 95\textsuperscript{th} percentile.
Supplement Figure 1

n=454
PEAR
European Ancestry

n=234
Atenolol Group

n=150
selected for metabolomic profiling based on BP response quartile

n=122
included in this analysis without IFG at baseline

n=24
developed IFG

n=98
did not develop IFG

n=233
included in genotyping analysis

n=184
included in this analysis without IFG at baseline

n=29
developed IFG

n=155
did not develop IFG

n=1 excluded due to genotype quality control issues
Supplement Figure 2

A

B

ISOLEUCINE
p=0.002

LEUCINE
p=0.011

VALINE
p=0.016

TYROSINE
p=0.22

PHENYLALANINE
p=0.036